

Hiroshi HARA* and Sachiko KUROSAWA*: **Cytotaxonomical
notes on some Asiatic species of *Agrimonia***

原 寛*・黒沢幸子*: キンミズヒキ類の細胞分類学的研究**

(Plates XX-XXI)

The Japanese plants of the genus *Agrimonia* (Rosaceae) were studied by Koidzumi and Nakai, and Koidzumi (1930) described *A. japonica* and *A. nipponica*, while Nakai (1933) regarded them as varieties of *A. pilosa* Ledeb. Since then the third species, *A. coreana* Nakai, was recorded from Japan. In recent years several Japanese botanists have revised the group, and Kitagawa (1963) and Ko. Ito (1963) recognized three species from Japan. Whereas Kitamura (1961) classified them in one species and one variety, and Ohwi recognized one species and one form in 1953, and two species in his latest work (1965).

We have been interested in Asiatic races of the group for several years, and the junior author has studied them cytologically. Now it became apparent that both tetraploid and octaploid races occur in Japan.

The plant widely distributed and very common in Japan has been called as *A. japonica* (Miq.) Koidzumi or *A. pilosa* var. *japonica* (Miq.) Nakai. The leaves are scattered throughout a tall stem which is often branching in the upper part. The larger leaflets of principal leaves are 5-9, rhombic oblong to rhombic ovate often acute at the apex with acutish teeth, hirsute especially on nerves beneath, and copiously white- or yellowish-glandular-dotted beneath. The stipules are often broadly falcate and acute at the apex. The bracts are glabrescent or minutely pilose. The flowers are 6-11 mm in diameter, with obovate or narrow obovate petals 2.5-6 mm long and 1.5-2 mm wide, and 8-15 stamens. The fruiting hypanthium is obconical, about 3 mm long and 3 mm across, and shortly pilose, surmounted by rings of ascending and spreading hooked bristles about 3 mm long. The plant is variable in

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hairiness, and the stems are often densely hirsute with spreading long hairs, but sometimes almost glabrous excepting the upper part (cf. f. *subglabra* Nakai 1933).

The junior author has cytologically examined several collections of this race, and all of them have 56 somatic chromosomes in root-tip cells (Table 1). Sokolovskaja (1960) reported the same number for *A. pilosa* from Sakhalin, but the plant of Sakhalin is identical with the Japanese one.

This Japanese race was formerly confounded with *A. Eupatoria* L. which occurs from Europe through Asia Minor and Caucasus east to Afghanistan and Kashmir, and also in North Africa. However, *A. Eupatoria* has leaves crowded on the lower half of usually simple stem, coarsely serrate leaflets which are densely villose and almost non-glandular beneath, long scapiform peduncle, dense spike with hirsute rachis, long-ciliate bracts, and larger densely hirsute fruiting hypanthium 4-5 mm across, and it is always tetraploid with 28 somatic chromosomes.

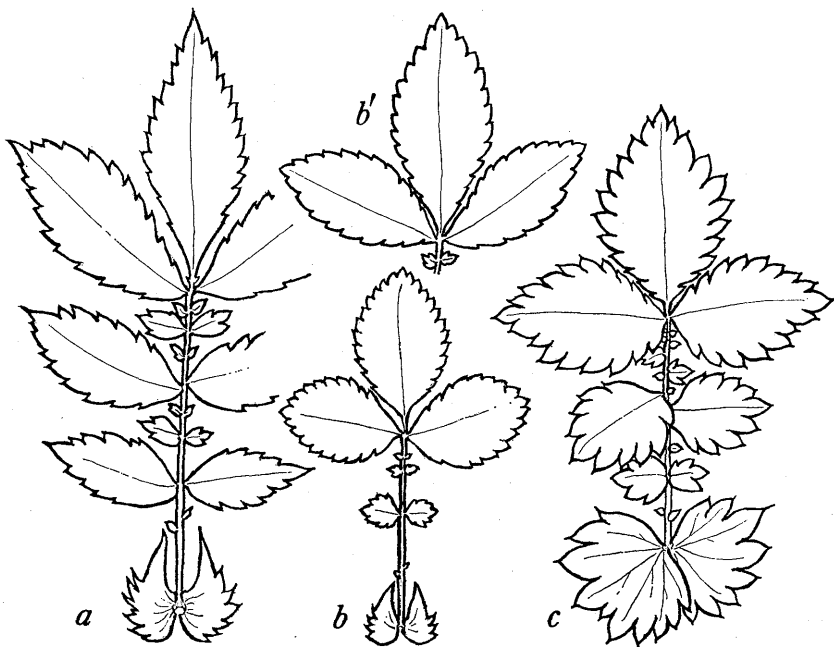


Fig. 1. Median leaves of *Agrimonia pilosa* ssp. *japonica* (a), *A. nipponica* (b, b'), and *A. coreana* (c). $\times 2/3$.

Table 1. Chromosome number.

Species	2n	Locality	Author
<i>A. Eupatoria</i>	28	Europe (England to Poland)	Various authors (see Löve 1961)
<i>A. repens</i>	28	S. Europe ?	Brittan 1953
<i>A. parviflora</i>	28	N. America. Virginia: Shenandoah National Park	Kurosawa
<i>A. nipponica</i>	28	Japan. Honshu (8 localities ¹⁾)	Kurosawa
	28	Japan. Kyushu. Is. Yakusima	Kurosawa
<i>A. coreana</i>	28	Japan. C. Honshu: Hachiôji, Shiroyama & Mt. Ibuki	Kurosawa
<i>A. pilosa</i>	28	No precise locality	Brittan 1953
ssp. <i>japonica</i>	56	Sakhalin & Primorsk	Sokolovskaja 1960 & 66
	56	Japan. Honshu (10 localities ²⁾)	Kurosawa
	56	Japan. Kyushu. Tsushima: Tsutsuzaki & Tatsura	Kurosawa
var. <i>nepalensis</i>	56	C. Nepal. Kathmandu, Gokarna (cult. in Tokyo)	Kurosawa
	56	E. Nepal. Akasay (cult. in Tokyo)	Kurosawa
<i>A. striata</i>	56	N. America	Brittan 1953
<i>A. grypsosepala</i>	56	N. America	Brittan 1953
<i>A. odorata</i>	56	Europe	Wulff 1939; Brittan 1953

1) Honshu. Towada, Oirase; Nasu, Itamuro; Karuizawa, Atagoyama, Usui-tôge, & Minamikaruizawa; Tokyo, Mt. Takao; Chiba, Nakamura; Ise, Naigu.

2) Honshu. Sendai; Nasu, Barappara; Karuizawa, Kôse, Usui-tôge & Minamikaruizawa, including a glabrescent form; Tokyo, Mt. Takao; Kai, Mitsutôge; Idzu, Hikanesan; Ômi, Mt. Ibuki; Iki, Shiine.

Another race which is also widely distributed in Japan is generally referred to *A. nipponica* Koidzumi. As compared with *A. japonica*, the race has often abbreviated lower internodes of the stem, 3-5 (7) elliptic or obovate leaflets of principal leaves less pointed at the apex with more roundish teeth and inconspicuously white-glandular beneath, slender spike, smaller flowers 5-7 mm in diameter, oblong petals 3-4 mm long 1-1.2 mm wide, 5 (-8) stamens, and smaller fruiting hypanthium 2 mm long, 2-3 mm across, and stiffly pilose on ridges, surmounted by fewer hooked bristles about 2 mm long. Higashi and others (1956) reported that clustered crystals of calcium oxalate were observed in leaf-tissues of *A. japonica*, while solitary crystals were found in those of

A. nipponica. Kitagawa and Ko. Ito (1963) have adopted this character in separating the two species. Now *A. nipponica* collected from several localities in Japan were proved to have $2n=28$ chromosomes by the junior author (Table 1). In the plants collected from Towada, a satellite was observed on one pair of submedian chromosomes. It often grows in the same district as *A. japonica*, but generally in more shady places forming a separate population from that of the latter which grows in more sunny places. *A. nipponica* is often smaller and has thinner leaves than those of *A. japonica*, but some plants of *A. nipponica* including the type specimen have a tall stem attaining to 80 cm high and thicker leaves. *A. nipponica* seems to correspond with *A. microcarpa* Wallroth of North America.

The third race, *A. coreana* Nakai, is very distinct in having densely *villose stems with soft spreading* long and short white *hairs*, comparatively long lower internodes, 3-5 (7) ovate or rhombic-ovate larger leaflets of principal leaves which are *obtusely serrate*, and villose, and inconspicuously gland-dotted beneath, *flabellate* spreading and coarsely toothed *stipules*, *loose elongate spike* long-interrupted in the lower part, densely villose rachis, smaller bracteoles, flowers 7-15 mm in diameter, oblong or elliptic petals 3.5-5 mm long and 1.6-2.5 mm wide, 12-28 *stamens*, and appressed hirsute fruiting *hypanthium* 3-4 mm long and 3-6 mm across with ascending bristles 2-2.5 mm long.

This species was first described from Middle Korea. It is growing very locally in open grassy places or on the edge of forest, and sometimes on limestone, and has been recorded from about 12 scattering localities from Hokkaido south to Kyushu¹⁾, and also from North China and Ussuri. The plants collected at Shiroyama of Hachiôji (Ongata-mura) near Tokyo by Mr. Tatuo Sato flower nearly a month earlier than the other races. The species was found to have 28 somatic chromosomes by the junior author (Table 1).

Some specimens of *A. coreana* resemble *A. nipponica* in the shape of leaves, but have softer spreading hairs on stems and leaves, longer lower internodes, spreading stipules, looser spikes, larger flowers with numerous stamens, and larger hirsute fruits with ascending bristles.

The three Japanese races mentioned above are distinguishable each other by morphological, cytogenetical and ecological characters, and can be regarded

1) Honshu: Mt. Ibuki, 1000-1300 m (Hara & Kurosawa, Aug. 23, 1968, TI). Kyushu. Prov. Hiuga: on limestone of Mt. Shiraiwa, 1600 m (S. Hirata no. 82, Sep. 26, 1953, TI).

as three separate species.

But, as suggested by Nakai (1933), *A. pilosa* Ledeb. (1823), *A. nepalensis* D. Don (1825), *A. viscidula* Bunge (1833), and *A. japonica* may be considered to belong to a single species.

Typical *A. pilosa* has generally simple stems, thin acutish leaflets which are cuneate at the base, sparsely hairy or glabrescent above, and hirsute on nerves and sparsely glandular beneath, fewer coarse leaf-teeth which are deep in the lower leaves, ciliate bracts, and fruits with erectly connivent bristles. It is distributed from Eastern Europe through Siberia east to North China, and was reported by Brittan to have $2n=28$ chromosomes.

Agrimonia japonica has much more densely pubescent and glandular leaflets with broadly cuneate at the base and numerous teeth, and fruits with spreading outer bristles. In morphological characters, however, intermediate forms between *A. pilosa* and *A. japonica* are sometimes found. It is still doubtful if typical *A. pilosa* from Altai is tetraploid, and detailed cytotaxonomical investigations on Siberian materials are much needed.

Regarding *A. nepalensis* D. Don, we have collected and studied living materials in Nepal during our Botanical Expeditions to Eastern Himalaya organized by the University of Tokyo. They have densely hirsute stems with spreading long and short hairs, 5-9 obovate or elliptic larger leaflets of principal leaves which are often roundish at the apex, and velutinous and glandular on both surfaces, pubescent bracts, flowers 7-9 mm across, elliptic petals 3.5-4.8 mm long and 1.2-1.8 mm wide, about 10 stamens, fruiting hypanthium 2.5-3 mm long and 3-4 mm across, with ascending and spreading bristles up to 2.5 mm long. Three collections from Nepal by our Expeditions now cultivated in Tokyo were cytologically examined, and they have $2n=56$ chromosomes (Table 1). They agree with *A. japonica* in the character of flowers and fruits and also in the chromosome number, but have generally more roundish leaflets and much more densely hirsute stems and leaves. However, a few specimens from Nepal have acutish leaflets like the Japanese ones, and the specimens from Western Himalaya coincide with the Japanese in the shape of leaflets and the hairiness. Judging from these facts, it seems better to regard the Japanese and the Nepalese as conspecific.

Agrimonia zeylanica Moon from Ceylon may also be conspecific with *A. nepalensis*, and at least some of the specimens from W. China and E. Bengal

Table 2. Pollen grains.

Species	Chrom.	Pollen size (μ)	Locality	Author
<i>A. nipponica</i>	4x	28-32 \times 22-27*	Karuizawa	Kurosawa
		30-35 \times 27-30*	Karuizawa	Kurosawa
		37-39 \times 22-24*	Chiba, Nakamura	Kurosawa
		29-36 \times 19-24*	Ise, Naigu	Kurosawa
<i>A. coreana</i>	4x	28-29 \times 19-20*	Hachiôji	Kurosawa
		34-36 \times 20	Nagano,	Kurosawa
			Mt. Hachibuse	
<i>A. pilosa</i> ssp. <i>japonica</i>	8x	34-36 \times 22-27*	Mt. Ibuki	Kurosawa
		34-39 \times 27-29*	Narashino	Ikuse 1956
			& Yakusima	
		31-34 \times 24-26*	Mt. Takao	Kurosawa
var. <i>nepalensis</i>	8x	34-36 \times 24-26*	Mitsutôge	Kurosawa
		34-36 \times 26-29	E. Nepal, Dhankuta	Kurosawa
		36-43 \times 27-31*	C. Nepal, Gokarna	Kurosawa

* Examined by living materials.

referred to *A. zeylanica* by Handel-Mazzetti (1933) seem to be identical with *A. nepalensis*. *A. viscidula* Bunge seems to be also conspecific with *A. japonica*.

The size of pollen grains of Asiatic species is shown in Table 2. The shape is subprolate or prolate, and it seems to vary in shape and size by individual to some extent. It is rather noteworthy that no remarkable differences are observed between species, and in this group the size of pollen grains is almost the same both in tetraploid and octaploid species and no apparent correlation between pollen-size and polyploidy could be detected. On the contrary, the pollen size of *A. Eupatoria*, a tetraploid species, is slightly larger than the other species.

The type of the crystals of calcium oxalate in leaf-tissue is also examined. In such octaploid plants as *A. pilosa* subsp. *japonica* and var. *nepalensis*, the crystals are all clustered. Whereas in tetraploid species such as *A. nipponica*, *A. coreana*, and also *A. parviflora* and *A. Eupatoria*, many solitary crystals are observed in leaf-tissue. So far the type of the crystals correlates with polyploidy in the most case. But it needs to be confirmed whether a race of *A. pilosa* reported to have 28 somatic chromosomes has solitary crystals or not.

It is certain that polyploidy has played an important role in speciation of the genus *Agrimonia* in Asia as well as in Europe and North America.

The Asiatic species discussed above are summarized as follows:

Agrimonia pilosa Ledeb., Ind. Hort. Dorpat. Suppl. 1 (1823)—Nakai in Bot. Mag. Tokyo 47: 244 (1933)—Kitamura et al., Col. Ill. Herb. Pl. Jap. 2: 122 (1961)—Ohwi, Fl. Jap. ed. rev. 758 (1965).

subsp. **pilosa**.

Distr. Eastern Europe, Siberia, and North China.

subsp. **japonica** (Miquel) Hara, stat. nov.

A. Blumei G. Don, Gen. Hist. 2: 563 (1832), quoad pl. jap.—Meyer in Bull. Sci. Acad. Sci. St.-Petersb. 10: 549 (1842), p. p.

A. viscidula var. *japonica* Miquel, Ann. Mus. Lugd.-Bat. 3: 38 (1867).

A. japonica (Miq.) Koidzumi in Bot. Mag. Tokyo 44: 104 (1930)—Juzep. in Fl. URSS 10: 419 (1941).

A. Eupatoria var. *japonica* (Miq.) Masamune in Ann. Rep. Taihoku Bot. Gard. 2: 134 (1932).

A. pilosa var. *japonica* (Miq.) Nakai in Bot. Mag. Tokyo 47: 245 (1933)—Hara in Bot. Mag. Tokyo 49: 122 (1935)—Ko. Ito in Hokuriku Journ. Bot. 12: 3 (1963).

Distr. Indo-China, China, Taiwan, Ussuri, Korea, Saghalin, S. Kuriles, and Japan.

var. **nepalensis** (D. Don) Nakai in Bot. Mag. Tokyo 47: 247 (1933).

A. nepalensis D. Don, Prodr. Fl. Nepal. 229 (1825)—Jacquem., Voyag. 4: t. 68 (1844).

A. Eupatoria γ. *nepalensis* (D. Don) O. Kuntze, Rev. Gen. Pl. 1: 214 (1891).

Distr. Himalayas, Indo-China, and China.

Agrimonia nipponica Koidzumi in Bot. Mag. Tokyo 44: 104 (1930)—Kitagawa in Journ. Jap. Bot. 33: 162 (1958); 38: 305 (1963)—Ko. Ito in Hokuriku Journ. Bot. 12: 4 (1963)—Ohwi, Fl. Jap. ed. rev. 759 (1965).

A. pilosa f. *nipponica* (Koidz.) Ohwi in Bull. Sci. Mus. Tokyo 33: 76 (1953).

A. pilosa var. *nipponica* (Koidz.) Kitamura in Acta Phyt. Geobot. 20: 199 (1962)—Ohwi, Fl. Jap. ed. eng. 539 (1965).

Distr. Japan (W. Hokkaido to Kyushu, incl. Yakusima), and Quelpaert.

Agrimonia coreana Nakai, Rep. Veg. Diamond Mts. 71, 72 & 175 (1918), cum diagn. jap. brev.; Kôryô-Shikenrin-no-Ippan 83 (1932)—Kitagawa, Lineam. Fl. Mansh. 257 (1939); in Journ. Jap. Bot. 38: 305 (1963)—Ko. Ito in Hokuriku

Journ. Bot. 12: 7 (1963).

A. velutina Juzepczuk in Fl. URSS. 10: 420, t. 25. 5 & 636 (1941).

A. pilosa var. *simplex* T. Shimizu in Acta Phyt. Geobot. 17: 87, f. 2 (1958).

A. tokatiensis Ko. Ito in Hokuriku Journ. Bot. 9: 69, fig. (1961).

Distr. Japan (S. Hokkaido to Kyushu), Korea, Manchuria, and Ussuri.

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Explanation of Plates XX-XXI

- Pl. XX. Figs. a-d. *Agrimonia pilosa* ssp. *japonica*. a. Inflorescence. $\times 3/2$. b. Clustered crystals of calcium oxalate in leaf-tissue. \times ca. 125. c. Infructescence. $\times 3/2$. d. Somatic chromosomes. $\times 1700$. e. Somatic chromosomes of var. *nepalensis*. $\times 1700$.
- Pl. XXI. Figs. a-d. *Agrimonia nipponica*. a. Inflorescence. $\times 3/2$. b. Solitary crystals of calcium oxalate in leaf-tissue. \times ca. 125. c. Infructescence. $\times 3/2$. d. Somatic chromosomes. $\times 1700$. Figs. e-g. *A. coreana*. e. Inflorescence. $\times 3/2$. f. Infructescence. $\times 3/2$. g. Somatic chromosomes. $\times 1700$.

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日本のキンミズヒキ類は小泉・中井両先生 (1930 & 33) によって検討されたが、近年再び論議され北川・伊藤浩司両博士 (1963) は 3 種を認められ、北村博士 (1961) は 1 種 1 変種を、また大井博士は初め (1953) 1 種 1 品種とされたが最近 (1965) は 2 種を確認されている。

日本で最も普通なキンミズヒキは古くはヨーロッパの *A. Eupatoria* と混同されたこともあったが形態的にかなりはっきり区別され、染色体数は *A. Eupatoria* は $2n=28$ であるがキンミズヒキは 56 であることが今回明らかになった。しかしキンミズヒキにもかなりの変異が見られ、茎には開出毛を密生するものが多いが、上部を除き無毛のものもある。小葉の形もかなり変わるが茎下部の葉を除き多くは鋭頭になり、下面には細腺点と脈上に毛が多い。果実の鉤刺は外側のものは平開するが終りには立つようになる。花卉は倒卵状で雄蕊は 8-15 本あり、群落によりまた同一花穂中でもある程度は変化する。2 本の花柱は不同長のことが多い。

ヒメキンミズヒキは北川博士 (1958) も指摘されたように形態的に区別でき、また染色体数は 28 でキンミズヒキと異なることが分った。分布は広が多く樹蔭に生じ、小葉の数が少く円味があり鋸齒も円味があって下面の腺点は目立たず、花は小さく花卉は長楕円形で雄蕊は 5-8 本、果実も一まわり小さい。ヒメキンミズヒキはキンミズヒキよりよく茎が細そく葉が軟質であるとされているが、丈が高く葉はやや厚質になったり、また小葉が鋭頭になることもある。

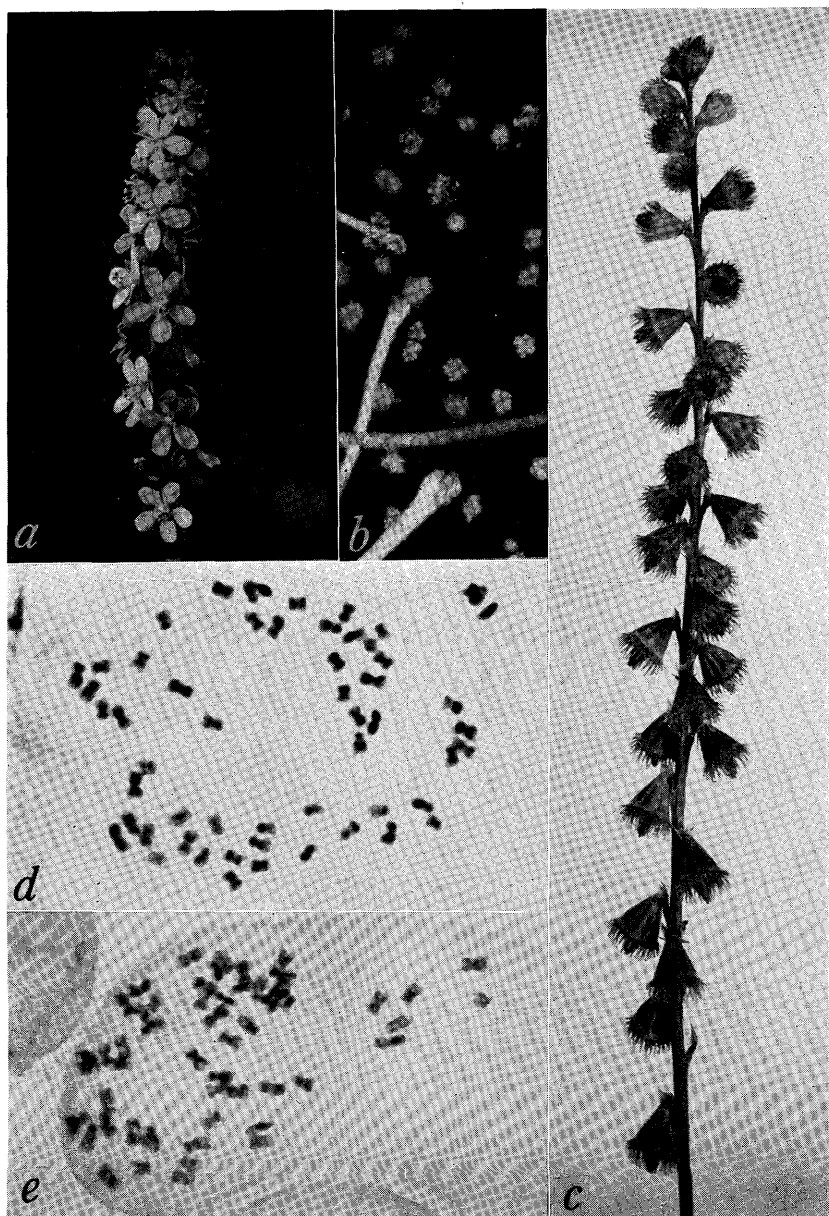
チョウセンキンミズヒキは日本では佐藤達夫氏の八王子 (恩方村) 城山の採品 (1943 & 49) を中井博士が同定されたのが最初で、渡嘉敷裕氏によって野草 24(5): 6 (1958) に詳しく書かれているが、後に北川博士 (1963) も同じ結論を出されている。生育地はごく局限されているが全体としては北海道から九州にまで点在し、往々石灰岩地に生える。茎・花軸や葉に長短の軟毛が密生しており、托葉は半円形で横に開き粗齒があり、花穂はまばらで花・果は大きく雄蕊の数も多い。染色体数は $2n=28$ である。

これら三者は形態的、生態的また細胞遺伝学的に分化していて別種とみなされるが、その間に雑種ができるかどうか今後の調査にまたなければならない。

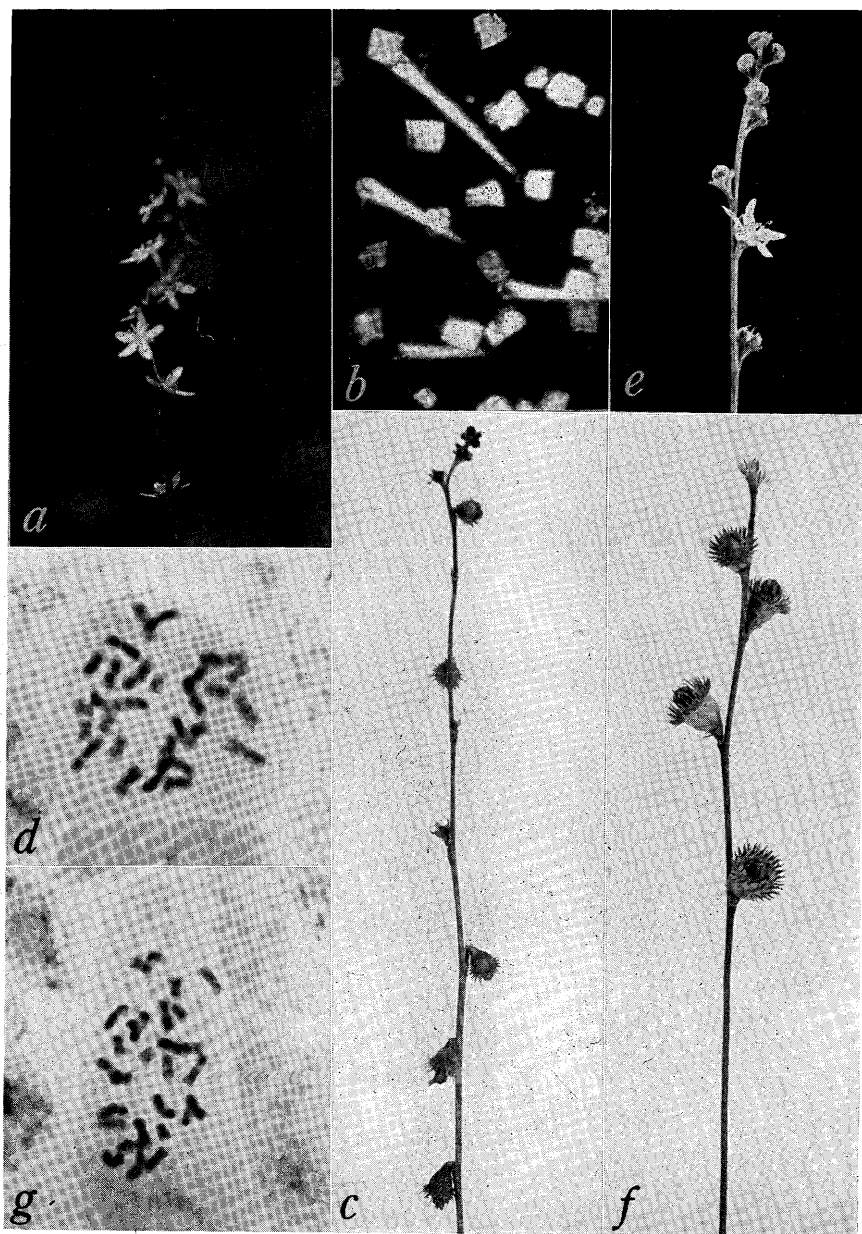
東部ヒマラヤ産のものは花や果はキンミズヒキに近く染色体数も $2n=56$ であるが、小葉は円味があり毛が多い。今後シベリヤや中国産のものについて細胞分類学的研究が必要である。この類では花粉粒の大きさ (表 2) は倍数性とあまり相関していない。

本属の種は外部形態的の差異は比較的小さいが、東亜でも北米やヨーロッパでも倍数性が種分化に大きな役割りを演じていることが分る。

終りに本研究資料の採集に協力して下さった佐藤達夫、山崎 敬、大橋広好、中馬千鶴の諸氏に深く謝意を表します。



HARA & KUROSAWA: Asiatic species of *Agrimonia*

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